

## What Is Claimed Is:

1. A shuffled DNA molecule, wherein said shuffled DNA molecule encodes a protox enzyme having enhanced tolerance to a herbicide that inhibits the protox activity encoded by a template DNA molecule from which said shuffled DNA molecule is derived.
2. The shuffled DNA molecule of claim 1, wherein said herbicide is selected from the group consisting of an aryluracil, a diphenylether, an oxidiazole, an imide, a phenyl pyrazole, a pyridyl pyrazole, a pyridine derivative, a 3-substituted-2-aryl-4,5,6,7-tetrahydroindazole, a phenopylate and O-phenylpyrrolidino- and piperidinocarbamate analogs of said phenopylate.
3. A mutagenized DNA molecule obtained by shuffling a template DNA molecule encoding an enzyme having protox activity, wherein said mutagenized DNA molecule encodes a protox enzyme having enhanced tolerance to a herbicide that inhibits the protox activity encoded by said template DNA molecule.
4. The mutagenized DNA molecule of claim 3, wherein said herbicide is selected from the group consisting of an aryluracil, a diphenylether, an oxidiazole, an imide, a phenyl pyrazole, a pyridyl pyrazole, a pyridine derivative, a 3-substituted-2-aryl-4,5,6,7-tetrahydroindazole, a phenopylate and O-phenylpyrrolidino- and piperidinocarbamate analogs of said phenopylate.
5. A method for forming a mutagenized DNA molecule encoding an enzyme having protox activity from a template DNA molecule encoding an enzyme having protox activity, wherein said template DNA molecule has been cleaved into double-stranded-random fragments, said method comprising the steps of:
  - a) adding to the resultant population of double-stranded-random fragments at least one single-stranded or double-stranded oligonucleotide, wherein said oligonucleotide comprises an area of identity and an area of heterology to the template DNA molecule;

- b) denaturing the resultant mixture of double-stranded-random fragments and oligonucleotides into single-stranded molecules;
- c) incubating the resultant population of single-stranded molecules with a polymerase under conditions that result in the annealing of said single-stranded molecules at said areas of identity to form pairs of annealed fragments, said areas of identity being sufficient for one member of a pair to prime replication of the other, thereby forming a mutagenized double-stranded polynucleotide;
- d) repeating steps b) and c) for at least two further cycles, wherein the resultant mixture in step b) of a further cycle includes the mutagenized double-stranded polynucleotide from step c) of the previous cycle, and the further cycle forms a further mutagenized double-stranded polynucleotide;

wherein the mutagenized double-stranded polynucleotide encodes a protox enzyme having enhanced tolerance to a herbicide that inhibits the protox activity encoded by the template DNA molecule.

- 6. The method of claim 5, wherein said template DNA molecule is derived from a eukaryote.
- 7. The method of claim 6, wherein said eukaryote is a higher eukaryote.
- 8. The method of claim 7, wherein said higher eukaryote is a plant.
- 9. The method of claim 8, wherein said plant is selected from the group consisting of *Arabidopsis thaliana*, oilseed rape, soybean, sugarbeet, cotton, maize, wheat, rice, sugarcane, and sorghum.
- 10. The method of claim 8, wherein said template DNA molecule derived from said plant comprises at least one mutation and encodes a modified protoporphyrinogen oxidase (protox) having at least one amino acid modification, wherein said modified protox is tolerant to a herbicide in amounts that inhibit said protox.

11. The method of claim 10, wherein wherein said template DNA molecule is further characterized in that at least one of the following conditions is met:

- (a) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $AP\Delta_1F$ , wherein  $\Delta_1$  is an amino acid other than arginine;
- (b) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $F\Delta_2S$ , wherein  $\Delta_2$  is an amino acid other than cysteine;
- (c) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $Y\Delta_3G$ , wherein  $\Delta_3$  is an amino acid other than alanine;
- (d) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $AA\Delta_4D$ , wherein  $\Delta_4$  is an amino acid other than glycine;
- (e) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $Y\Delta_5P$ , wherein  $\Delta_5$  is an amino acid other than proline;
- (f) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $P\Delta_6A$ , wherein  $\Delta_6$  is an amino acid other than valine;
- (g) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $\Delta_7IG$ , wherein  $\Delta_7$  is an amino acid other than tyrosine;
- (h) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $YIGG\Delta_8$ , wherein  $\Delta_8$  is an amino acid other than alanine or serine;
- (i) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $A\Delta_9P$ , wherein  $\Delta_9$  is an amino acid other than isoleucine;
- (j) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $G\Delta_{10}A$ , wherein  $\Delta_{10}$  is an amino acid other than valine;
- (k) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $Y\Delta_3G$ , wherein  $\Delta_3$  is an amino acid other than alanine, and said template DNA molecule also has a sequence that encodes one of the group consisting of:
  - (1) sub-sequence  $Q\Delta_{11}S$ , wherein  $\Delta_{11}$  is an amino acid other than proline,
  - (2) sub-sequence  $IGG\Delta_{12}$ , wherein  $\Delta_{12}$  is an amino acid other than threonine,
  - (3) sub-sequence  $SWXL\Delta_{13}$ , wherein  $\Delta_{13}$  is an amino acid other than serine,
  - (4) sub-sequence  $L\Delta_{14}Y$ , wherein  $\Delta_{14}$  is an amino acid other than asparagine,
 and

- (5) sub-sequence  $G\Delta_{15}XGL$ , wherein  $\Delta_{15}$  is an amino acid other than tyrosine;
- (l) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $\Delta_7IG$ , wherein  $\Delta_7$  is an amino acid other than tyrosine, and said template DNA molecule also has a sequence that encodes one of the group consisting of:
- (1) sub-sequence  $Q\Delta_{11}S$ , wherein  $\Delta_{11}$  is an amino acid other than proline,
  - (2) sub-sequence  $IGG\Delta_{12}$ , wherein  $\Delta_{12}$  is an amino acid other than threonine,
  - (3) sub-sequence  $SWXL\Delta_{13}$ , wherein  $\Delta_{13}$  is an amino acid other than serine,
  - (4) sub-sequence  $L\Delta_{14}Y$ , wherein  $\Delta_{14}$  is an amino acid other than asparagine, and
  - (5) sub-sequence  $G\Delta_{15}XGL$ , wherein  $\Delta_{15}$  is an amino acid other than tyrosine,
- and
- (m) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $T\Delta_{16}G$ , wherein  $\Delta_{16}$  is an amino acid other than leucine, and said template DNA molecule also has a sequence that encodes amino acid sub-sequence  $YV\Delta_{17}G$ , wherein  $\Delta_{16}$  is an amino acid other than alanine.
12. The method of claim 5, wherein said template DNA molecule is derived from a prokaryote.
13. The method of claim 5, wherein said herbicide is selected from the group consisting of an aryluracil, a diphenylether, an oxidiazole, an imide, a phenyl pyrazole, a pyridyl pyrazole, a pyridine derivative, a 3-substituted-2-aryl-4,5,6,7-tetrahydroindazole, a phenopylate and O-phenylpyrrolidino- and piperidinocarbamate analogs of said phenopylate.
14. A mutagenized DNA molecule encoding an enzyme having protox activity obtained by the method of claim 5, wherein said mutagenized DNA molecule encodes a protox enzyme having enhanced tolerance to a herbicide that inhibits the protox activity encoded by said template DNA molecule.

1 ~~15~~. A method for forming a mutagenized DNA molecule encoding an enzyme having protox activity from at least two non-identical template DNA molecules encoding enzymes having protox activity, said method comprising the steps of:

- a) adding to the template DNA molecules at least one oligonucleotide comprising an area of identity to each of the template DNA molecule;
- b) denaturing the resultant mixture into single-stranded molecules;
- c) incubating the resultant population of single-stranded molecules with a polymerase under conditions that result in the annealing of the oligonucleotides to the template DNA molecules, wherein the conditions for polymerization by the polymerase are such that polymerization products corresponding to a portion of the template DNA molecules are obtained;
- d) repeating the second and third steps for at least two further cycles, wherein the extension products obtained in step c) are able to switch template DNA molecule for polymerization in the next cycle, thereby forming a mutagenized double-stranded polynucleotide comprising sequences derived from different template DNA molecules;

wherein the mutagenized double-stranded polynucleotide encodes a protox enzyme having enhanced tolerance to a herbicide that inhibits the protox activity encoded by the template DNA molecules.

2 ~~16~~. The method of claim <sup>1</sup>~~15~~, wherein at least one template DNA molecule is derived from a eukaryote.

3 ~~17~~. The method of claim <sup>2</sup>~~16~~, wherein said eukaryote is a higher eukaryote.

4 ~~18~~. The method of claim <sup>3</sup>~~17~~, wherein said higher eukaryote is a plant.

5 ~~19~~. The method of claim <sup>3</sup>~~17~~, wherein said plant is selected from the group consisting of *Arabidopsis thaliana*, oilseed rape, soybean, sugarbeet, cotton, maize, wheat, rice, sugarcane, and sorghum.

6 20. The method of claim <sup>3</sup>17, wherein at least one said template DNA molecule derived from said plant comprises at least one mutation and encodes a modified protoporphyrinogen oxidase (protox) having at least one amino acid modification, wherein said modified protox is tolerant to a herbicide in amounts that inhibit said protox.

21. The method of claim 20, wherein wherein at least one said template DNA molecule is further characterized in that at least one of the following conditions is met:

- (a) said template DNA molecule has a sequence that encodes amino acid subsequence  $AP\Delta_1F$ , wherein  $\Delta_1$  is an amino acid other than arginine;
- (b) said template DNA molecule has a sequence that encodes amino acid subsequence  $F\Delta_2S$ , wherein  $\Delta_2$  is an amino acid other than cysteine;
- (c) said template DNA molecule has a sequence that encodes amino acid subsequence  $Y\Delta_3G$ , wherein  $\Delta_3$  is an amino acid other than alanine;
- (d) said template DNA molecule has a sequence that encodes amino acid subsequence  $AA\Delta_4D$ , wherein  $\Delta_4$  is an amino acid other than glycine;
- (e) said template DNA molecule has a sequence that encodes amino acid subsequence  $Y\Delta_5P$ , wherein  $\Delta_5$  is an amino acid other than proline;
- (f) said template DNA molecule has a sequence that encodes amino acid subsequence  $P\Delta_6A$ , wherein  $\Delta_6$  is an amino acid other than valine;
- (g) said template DNA molecule has a sequence that encodes amino acid subsequence  $\Delta_7IG$ , wherein  $\Delta_7$  is an amino acid other than tyrosine;
- (h) said template DNA molecule has a sequence that encodes amino acid subsequence  $YIGG\Delta_8$ , wherein  $\Delta_8$  is an amino acid other than alanine or serine;
- (i) said template DNA molecule has a sequence that encodes amino acid subsequence  $AA\Delta_9P$ , wherein  $\Delta_9$  is an amino acid other than isoleucine;
- (j) said template DNA molecule has a sequence that encodes amino acid subsequence  $G\Delta_{10}A$ , wherein  $\Delta_{10}$  is an amino acid other than valine;

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- (k) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $Y\Delta_3G$ , wherein  $\Delta_3$  is an amino acid other than alanine, and said template DNA molecule also has a sequence that encodes one of the group consisting of:
- (1) sub-sequence  $Q\Delta_{11}S$ , wherein  $\Delta_{11}$  is an amino acid other than proline,
  - (2) sub-sequence  $IGG\Delta_{12}$ , wherein  $\Delta_{12}$  is an amino acid other than threonine,
  - (3) sub-sequence  $SWXL\Delta_{13}$ , wherein  $\Delta_{13}$  is an amino acid other than serine,
  - (4) sub-sequence  $L\Delta_{14}Y$ , wherein  $\Delta_{14}$  is an amino acid other than asparagine, and
  - (5) sub-sequence  $G\Delta_{15}XGL$ , wherein  $\Delta_{15}$  is an amino acid other than tyrosine;
- (l) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $\Delta_7IG$ , wherein  $\Delta_7$  is an amino acid other than tyrosine, and said template DNA molecule also has a sequence that encodes one of the group consisting of:
- (1) sub-sequence  $Q\Delta_{11}S$ , wherein  $\Delta_{11}$  is an amino acid other than proline,
  - (2) sub-sequence  $IGG\Delta_{12}$ , wherein  $\Delta_{12}$  is an amino acid other than threonine,
  - (3) sub-sequence  $SWXL\Delta_{13}$ , wherein  $\Delta_{13}$  is an amino acid other than serine,
  - (4) sub-sequence  $L\Delta_{14}Y$ , wherein  $\Delta_{14}$  is an amino acid other than asparagine, and
  - (5) sub-sequence  $G\Delta_{15}XGL$ , wherein  $\Delta_{15}$  is an amino acid other than tyrosine;
- (m) said template DNA molecule has a sequence that encodes amino acid sub-sequence  $T\Delta_{16}G$ , wherein  $\Delta_{16}$  is an amino acid other than leucine, and said template DNA molecule also has a sequence that encodes amino acid sub-sequence  $YV\Delta_{17}G$ , wherein  $\Delta_{17}$  is an amino acid other than alanine.

8 ~~22~~ The method of claim ~~15~~, wherein at least one said template DNA molecule is derived from a prokaryote.

9 ~~23~~ The method of claim ~~15~~, wherein said herbicide is selected from the group consisting of an aryluracil, a diphenylether, an oxidiazole, an imide, a phenyl pyrazole, a pyridyl pyrazole, a

pyridine derivative, a 3-substituted-2-aryl-4,5,6,7-tetrahydroindazole, a phenopylate and O-phenylpyrrolidino- and piperidinocarbamate analogs of said phenopylate.

24. A mutagenized DNA molecule encoding an enzyme having protox activity obtained by the method of claim 15, wherein said mutagenized DNA molecule encodes a protox enzyme having enhanced tolerance to a herbicide that inhibits the protox activity encoded by said template DNA molecule.

25. The method of claim 15, wherein said herbicide is selected from the group consisting of an aryluracil, a diphenylether, an oxidiazole, an imide, a phenyl pyrazole, a pyridyl pyrazole, a pyridine derivative, a 3-substituted-2-aryl-4,5,6,7-tetrahydroindazole, a phenopylate and O-phenylpyrrolidino- and piperidinocarbamate analogs of said phenopylate.

26. An isolated DNA fragment capable of specifically hybridizing to a eukaryotic protoporphyrinogen oxidase gene or mRNA, wherein said DNA fragment comprises a contiguous portion of a coding sequence for a protoporphyrinogen oxidase from a eukaryote at least 10 nucleotides in length.

27. The isolated DNA fragment of claim 26, wherein said coding sequence is selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, and 36.

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